Effect of asparagin on absorption and growth in wheat*

J. J. SKINNER AND J. H. BEATTIE

(WITH PLATE 33)

For the past few years a survey of the organic matter of the soil from the standpoint of biochemistry, has been under way in the Laboratory of Soil Fertility Investigations. As many as 25 different organic compounds have been isolated and identified. The definite recognition of these compounds has led to a fuller understanding of the chemistry of the organic matter of soils and of the biological changes taking place therein. The compounds isolated cover a wide range of chemical substances of biological origin. They are represented by the hydrocarbons, consisting of carbon and hydrogen only; by the acids, fats, resins, alcohols, esters, waxes, consisting of carbon, hydrogen, and oxygen; as well as by a large group of nitrogenous compounds, which consist of carbon, hydrogen, oxygen, and nitrogen.

The nitrogenous soil constituents isolated are creatinin,† with the probability that creatin also exists, hypoxanthin, xanthin, guanin, adenin, cholin, histidin, arginin,‡ nucleic acid,§ and picolin carboxylic acid.‖ A number of these have been studied in regard to their effect on plant growth. Some of the soil constituents have been found to be harmful as for instance, picolin

* Contribution from the Laboratory of Soil Fertility Investigations. Published by permission of the Secretary of Agriculture.

† Shorey, Edmund C. The isolation of creatinine from soils. Jour. Amer. Chem. Soc. 34: 99. 1912; also U. S. Dept. Agr. Bur. Soils Bull. 83[1]: 11–22. 1912.

‡ Schreiner, O., and Shorey, Edmund C. Chemical nature of soil organic matter. U. S. Dept. Agr. Bur. Soils Bull. 74: 34–36. 1910; The presence of arginine and histidine in soils. Jour. Biol. Chem. 8: 381. 1910; Pyrimidine derivatives and purine bases in soils. Jour. Biol. Chem. 8: 385. 1910.

§ Shorey, Edmund C. Nucleic acid in soils. Science II. 35: 390. 1912; Biochemical Bull. 1: 104. 1911.

‖ Schreiner, O., and Shorey, Edmund C. The isolation of picoline carboxylic acid from soils and its relation to soil fertility. Jour. Amer. Chem. Soc. 30: 1295. 1908; The isolation of harmful organic substances from soil. U. S. Dept. Agr. Bur. Soils Bull. 53: 1–53. 1909.

carboxylic acid|| and dihydroxystearic acid,* while others are beneficial to growth. Creatinin, one of the nitrogenous soil constituents, has a beneficial effect on growth.† An extended study has been reported on, with this compound. Plants grown in solution cultures containing only potash and phosphate show greatly increased growth when creatinin is added. When large amounts of nitrate are present in the culture solutions, creatinin produced no appreciable effect on the growth. Plants growing in nitrate cultures, whether low or high in nitrate, showed a greatly diminished absorption of this ingredient when creatinin was present, whereas the removal of potash and phosphate was practically normal. It seems that creatinin was absorbed by the plants, replacing the effect of nitrates. Creatin, nucleic acid,‡ hypoxanthin, and xanthin are also beneficial and have a similar action.

Many observations have been made with nitrogenous compounds not yet isolated from soils, although allied to the compounds which have been found to exist in soils. In this connection asparagin has been studied.

Asparagin is a water-soluble form of organic material which is relatively abundant in plants. It was first found in the young shoots of asparagus and subsequently in a large number of other plants representing many different families.

A number of investigators have worked with asparagin. Baessler§ found it to be beneficial to maize, Prianischnikoff and Lebedeff|| secured beneficial results, working with oats; Hansteen¶

* Schreiner, O., and Shorey, Edmund C. The isolation of dihydroxystearic acid from soils. Jour. Amer. Chem. Soc. **30**: 1599. 1908. Schreiner, O., and Skinner, J. J. Some effects of a harmful organic soil constituent. Bot. Gaz. **50**: 161. 1910; U. S. Dept. Agr. Bur. Soils Bull. 70: 1–98. 1910.

† Skinner, J. J. Effects of creatinine on growth and absorption. U. S. Dept. Agr. Bur. Soils Bull. 83^3: 33–41. 1911; Beneficial effect of creatinine and creatine on growth. Bot. Gaz. **54**: 152–163. *f. 1.* 16 Au 1912.

‡ Schreiner, O., and Skinner, J. J. The action of nucleic acid and its decomposition products on soils and plants. Science II. **35**: 390. 1912.

§ Baessler, P. Assimilation des Asparagins durch die Pflanze. Landw. Vers. Stat. **33**: 231. 1887.

|| Prianischnikoff, D., and Lebedeff, A. N. Assimilation of the nitrogen of some compounds in sterilized media. Tzv. Moscow Selskokhar Inst. **3**: 56. 1897; Abst. Exp. Sta. Rec. **9**: 820. 1907–8.

¶ Hansteen, B. Om aeggehvidesynthese i den grönne phanerogame plante. Vid. Selsk. Skrifter No. 3. 1898; Über Eiweissynthese in grünen Phanerogamen. Jahrb. Bot. **33**: 417. 1899.

found it was beneficial to *Lemna;* Brown* found it beneficial to the barley embryo; Nakamura† secured beneficial results, working with the barley plant, and also found it beneficial to onions; and Molliard ‡ secured beneficial results with radish. All of these tests were made in water cultures.

EXPERIMENTAL METHODS

In studying the effect of asparagin on growth, wheat seedlings were grown in aqueous culture solutions containing the nutrient salts, calcium acid phosphate, sodium nitrate, and potassium sulphate. Some of the cultures contained each of the salts singly, others were composed of a mixture of two salts, sodium nitrate and calcium acid phosphate, sodium nitrate and potassium sulphate, and calcium acid phosphate and potassium sulphate. Still other solutions had all three constituents in various proportions. In all there were 66 different cultures of nutrient solutions. The scheme of the experiment and manner of preparing the nutrient cultures was similar to that described in Bulletin 70 of the Bureau of Soils and other publications.§ The concentration of all these solutions was 80 parts per million of the fertilizer ingredients, P_2O_5, NH_3, and K_2O. In cultures containing only one nutrient salt, for instance calcium acid phosphate, the concentration was 80 parts per million of P_2O_5. If two salts were present, for instance calcium acid phosphate and sodium nitrate, the concentration was 80 parts per million of $P_2O_5 + NH_3$. If all three salts were present the concentration was 80 parts per million of $P_2O_5 + NH_3 + K_2O$. The ratios of these concentrations varied in 10 per cent stages, making in all 66 different cultures. Distilled water treated with carbon was used in preparing the culture solutions. The culture solutions

* Brown, H. T. On the culture of excised embryos of barley on nutrient solutions containing nitrogen in different forms. Trans. Guinness Research Lab. 1: 288. 1906.

† Nakamura, T. Relative value of asparagin as a nutrient for phanerogams. Bull. Coll. Agr. Tokyo 2: 465. 1894.

‡ Molliard, M. Recherches sur l'utilisation par les plantes supérieures de diverses substances organiques azotées. Bull. Soc. Bot. France IV. 10: 541. 1910.

§ Schreiner, O., and Skinner, J. J. Some effects of a harmful organic soil constituent. U. S. Dept. Agr. Bur. Soils Bull. 70: 1–98. 1910; Bot. Gaz. 50: 161. 1910; Ratio of phosphate, nitrate and potassium on absorption and growth. Bot. Gaz. 50: 1. 1910.

were contained in wide-mouth bottles holding 250 c.c., and 10 wheat plants were grown in each. The wheat plants when used for the experiment were about 2 cm. high; they had been previously germinated on aluminum disks floated upon the surface of a tank of water. When large enough to use in the experiment, seedlings of uniform size were selected for the test. The culture solutions were changed every three days, four changes being made during the course of the experiment. The solutions were analyzed for nitrates immediately after each change. The phosphate and potash were determined in a composite of the four changes. By this means the effect of asparagin upon the absorption of nutrients by the seedlings could be studied during the course of the experiment.

EFFECT OF ASPARAGIN ON GROWTH

Two sets of cultures were prepared: to one set were added merely the nutrient salts; to a similar set 50 parts per million of asparagin were added in each culture, in addition to the nutrient salts. The wheat seedlings grew in the culture solutions from November 13 to November 25.

When the plants had grown for several days it was noticeable that the asparagin cultures were better developed, these seedlings having broader leaves and longer and better developed roots. This was more pronounced in some of the fertilizer mixtures than in others. The beneficial effect became more decided as the experiment progressed. The weight of the plants taken at the end of the experiment shows the beneficial effect produced by the asparagin. The total green weight of the 66 cultures containing asparagin was 148.2 grams against 134.9 grams for the 66 control cultures, an increase of 9 per cent, as an average of all the asparagin cultures.

EFFECT OF ASPARAGIN ON GROWTH IN CULTURES CONTAINING NO NITRATE

The effect of the asparagin was more marked in the cultures containing potash and phosphate than in those which contained potash, phosphate, and nitrate. The green weight of the cultures composed of potash and phosphate with and without asparagin,

taken at the termination of the experiment, are given in TABLE I. The first column gives the number of the culture; the second, third, and fourth the amount of the fertilizer ingredient in the culture solution. Calcium acid phosphate, sodium nitrate, and potassium sulphate were the nutrient salts used. The fifth column gives the green weight of the plant without asparagin, and the sixth column the green weight with 50 parts per million of asparagin in the solution.

TABLE I

EFFECT OF ASPARAGIN ON GROWTH IN CULTURE SOLUTIONS CONTAINING NO NITRATE

Culture no.	Fertilizer ingredients in culture solution			Green weight of cultures	
	P_2O_5, parts per million	NH_3, parts per million	K_2O, parts per million	Without asparagin, grams	With 50 p. p. m. asparagin, gms.
56	0	0	80	1.385	1.832
46	8	0	72	1.368	1.644
37	16	0	64	1.382	1.742
29	24	0	56	1.119	1.825
22	32	0	48	1.393	2.145
16	40	0	40	1.223	2.199
11	48	0	32	1.167	1.932
7	56	0	24	1.243	2.199
4	64	0	16	1.313	1.909
2	72	0	8	1.311	1.775
1	80	0	0	0.811	0.975

All of the cultures with asparagin present show a marked increase in weight. Culture no. 37 containing 16 parts per million of phosphate and 64 parts per million of potash gave 1.382 grams green weight without asparagin and 1.742 grams with asparagin; culture no. 16, which contained 40 parts per million both of phosphate and of potash gave 1.223 grams weight without asparagin and 2.199 grams with asparagin. Culture no. 4, composed of 64 parts per million phosphate and 16 parts per million potash, gave 1.313 grams green weight without asparagin and 1.909 with asparagin. The total weight of the 11 cultures without asparagin was 13.714 grams against 20.478 grams with asparagin, thus showing an increase of 47 per cent as an average effect in the various cultures without nitrate. This series of cultures is reproduced in PLATE 33. Cultures marked with the same number, for instance 56 and 56*A*, have the same proportion of potash and phosphate. Cultures marked with the number alone contain no asparagin, the cultures with the letter *A* have 50 parts per million of asparagin. The composition of the culture solution is given in TABLE I. It will

be seen that each culture containing asparagin, no matter what the proportion of phosphate and potash may be, is larger than the culture growing in a similar solution without the asparagin.

EFFECT OF ASPARAGIN IN CULTURES CONTAINING NITRATE

In TABLE II are given the green weights obtained in the series of cultures, all of which contain the uniform amount of 8 parts per million NH_3 as nitrate, with varying amounts of potash and

TABLE II

EFFECT OF ASPARAGIN ON GROWTH IN CULTURE SOLUTIONS CONTAINING 8 PARTS PER MILLION NH_3 AS NITRATE

Culture no.	Fertilizer ingredients in culture solution			Green weight of cultures	
	P_2O_5, parts per million	NH_3, parts per million	K_2O, parts per million	Without asparagin, grams	With 50 p. p. m. asparagin, gms.
57	0	8	72	1.880	1.830
47	8	8	64	1.912	2.275
38	16	8	56	2.234	2.184
30	24	8	48	2.244	2.580
23	32	8	40	2.027	2.665
17	40	8	32	1.839	2.433
12	48	8	24	1.961	2.545
8	56	8	16	2.073	2.240
5	64	8	8	1.863	2.068
3	72	8	0	1.265	1.255

phosphate. The effect of asparagin is still noticeable in these green weight figures, as it was while the plants were growing, but the additional effect is much less marked than in the absence of nitrate as shown in TABLE I. The total growth in this series with-

TABLE III

EFFECT OF ASPARAGIN ON GROWTH IN CULTURE SOLUTIONS CONTAINING 16 PARTS PER MILLION NH_3 AS NITRATE

Culture no.	Fertilizer ingredients in culture solution			Green weight of cultures	
	P_2O_5, parts per million	NH_3, parts per million	K_2O, parts per million	Without asparagin, grams	With 50 p. p. m. asparagin, gms.
58	0	16	64	1.905	2.379
48	8	16	56	2.270	2.489
39	16	16	48	2.510	2.255
31	24	16	40	2.518	2.795
24	32	16	32	2.289	2.660
18	40	16	24	2.633	2.566
13	48	16	16	2.256	2.545
9	56	16	8	2.219	2.245
6	64	16	0	1.317	1.319

out asparagin was 19.298 grams against 22.075 grams with asparagin, an additional increase of 14 per cent due to asparagin. When no nitrate was present this additional effect of the asparagin was 47 per cent.

In TABLE III the green weight for the series of cultures containing 16 parts per million NH_3 as nitrate, is given. Without the asparagin the total weight of these cultures is 19.917 grams; with asparagin it is 21.253 grams, or only an increase of 7 per cent. The additional effect of asparagin was still less in the 24 parts per million NH_3 cultures, and with the higher amounts of nitrate this effect became even uncertain.

The effect of asparagin was much more pronounced in those fertilizer combinations that contained no nitrate and those low in nitrate. From this it appears that asparagin, like creatinin, creatin, hypoxanthin, xanthin, and a number of other nitrogenous compounds, can replace the effect of nitrate in producing plant growth.

INFLUENCE OF ASPARAGIN ON ABSORPTION OF FERTILIZER SALTS

The foregoing discussion shows clearly the influence of asparagin on growth and its effect in cultures containing no nitrate. There remains to be discussed the removal of nutrients from the solution during the growth of the plant. As already mentioned, the absorption of nutrients was determined by making an analysis for nitrate at the termination of every three-day change, and of the phosphate and potassium on a composite of the solution from the four changes. It is thus possible to compare the results obtained under the controlled conditions, without the asparagin and under the conditions where 50 parts per million of asparagin were present in the solution.

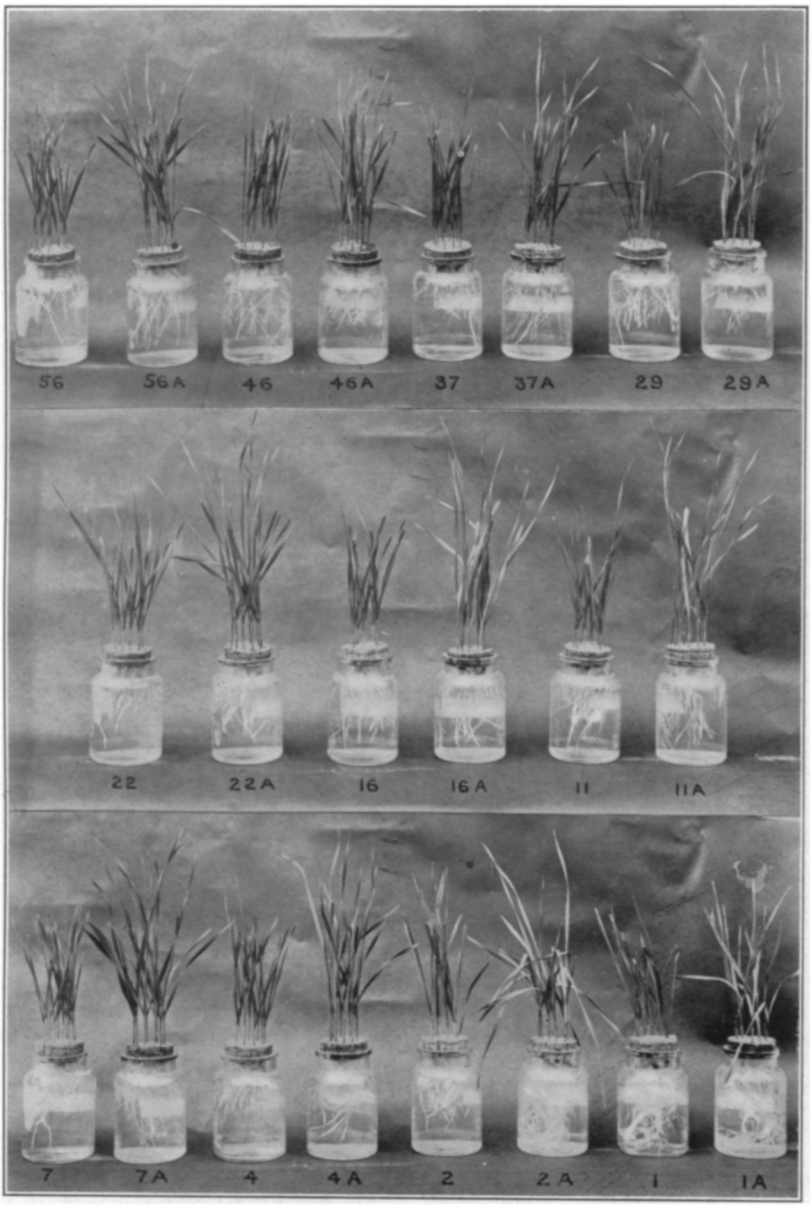
The total phosphate, potash, and nitrate removed from the cultures was 1,109.6 milligrams for the normal and 1,117 milligrams for the cultures containing asparagin. The examination of the results, when considered for the three constituents separately as given below, shows that the phosphate and potash absorption were somewhat greater in the asparagin cultures, as is demanded by the larger growth, whereas the nitrate removal is considerably less than in the normal cultures.

The amount of phosphate stated as P_2O_5 removed from the solutions during the experiment was 201.2 milligrams for the normal cultures and 326 milligrams for the cultures containing asparagin, a difference of 124.8 milligrams in favor of the asparagin cultures.

The amount of potash stated as K_2O removed from the solutions was 471.2 milligrams for the normal cultures and 485.6 milligrams for the asparagin cultures. As with the phosphate the asparagin cultures removed slightly more potash than the normal cultures, 14.4 milligrams.

The amount of nitrate removed during the course of the experiment was 437.2 milligrams by the normal and 305.4 milligrams for the asparagin cultures. The asparagin cultures, though making a larger growth, used 131.8 milligrams less nitrate. It seems that the plants absorb and use asparagin whether nitrate be present or not, the effect on growth being much more marked in the limiting case where no nitrate was present, and that in the other cases the compounds replaced the effect of nitrate.

The culture work was throughout under strict chemical control, so as to establish as definitely as possible that the effects on the plants noted were produced by the absorption of the compound as such. Nitrite, nitrate, and ammonia were tested for and found to be absent, or, in the case of ammonia, present in traces only. Although neither nitrate, nitrite, nor ammonia was found, the plants, nevertheless, grew remarkably well, and the only conclusion justified by this experimental evidence is that this compound is directly absorbed and assimilated. With the strict chemical control exercised, all possibility of any extended action by bacterial or other external biological agencies seems excluded. If such effects were produced in these experiments, they were of only minor significance in the results obtained. Bacteria and other microorganisms were excluded so far as possible, but no special effort was made to maintain absolutely sterile conditions, inasmuch as this would have been a practical impossibility in experiments on so large a scale, involving over a thousand plants in a single test. Moreover, it may even appear questionable whether absolute sterility, as being too artificial a condition for the determination of the effect of soil constituents on plants, would be desirable.



EFFECT OF ASPARAGIN ON WHEAT PLANTS

It would seem that chemical control under as normal conditions as a cultural experiment will allow, is better than conducting the experiment under the artificial condition of sterility, which, after all, is made only so that biochemical changes may be excluded. In these experiments the bottles were sterilized before being used in making culture solutions for the various changes, the pans and other apparatus used in germinating the seed were sterilized from time to time, and corks used for the cultures were always clean and sterilized before use. Although all of these precautions were taken, it was of course not possible to exclude some microorganisms in such work, as the solutions were exposed from time to time to the air. There was no excessive microorganic life noticeable. While bacteria and other microorganisms were present in the solutions to a slight extent, it can hardly be said that their influence could have been large; that is, such influence as they had was probably so slight as to be negligible so far as the general and larger tendencies which are shown to exist are concerned.

While the effect of the asparagin decreased with increasing nitrate so far as additional effect on growth is concerned, it had nevertheless a conserving effect upon the amount of nitrate left in the solution during the time the plants were growing, as is shown by the analysis of the solution. It appears, therefore, that the plant can utilize this nitrogenous compound for plant synthesis.

BUREAU OF SOILS,
WASHINGTON, D. C.

Explanation of plate 33

Wheat plants growing in culture solutions with and without asparagin, and containing various proportions of potash and phosphate but no nitrate. Cultures with the letter *A* following the number contain asparagin.